

Claims

1. A process for the production of a modified phytase having a desired property improved over the property of the corresponding unmodified phytase which comprises :

- 5 (a)determining the three dimensional structure of the unmodified phytase and of a second phytase which has the desired property by aligning the amino acid sequences of said phytases with the amino acid sequence of a third phytase which is the phytase of *Aspergillus niger* and using the three dimensional structure of the phytase of *Aspergillus niger* as a
10 template based on the alignment to determine said three dimensional structures;
- (b)determining from the structures of step (a) the amino acids of the active sites of the unmodified phytase and of the second phytase having the desired property which active site provides the desired property and
15 comparing the amino acids which form the active sites to identify which amino acids are different in the active site of the second phytase from the amino acids in the active site of the unmodified phytase;
- (c)constructing a DNA sequence coding for the modified phytase by obtaining the DNA sequence of the unmodified phytase and changing
20 the nucleotides coding for the active site which provides the desired property for said unmodified phytase so that at least one of the amino acids in the active site which provides the desired property is substituted by one of the amino acids which was identified as being different in step (b);
- 25 (d)integrating such a DNA sequence into a vector capable of expression in a suitable host cell; and
- (e)transforming the suitable host cell by the DNA sequence of step (c) or the vector of step (d), growing said host cell under suitable growth conditions and isolating the modified phytase from the host cell or the
30 culture medium.

2. The process of claim 1 wherein the unmodified phytase is of eukaryotic origin.

3. The process of claim 2 wherein the unmodified phytase is of fungal origin.

5 4. The process of claim 3 wherein the unmodified phytase is of *Aspergillus* origin.

5. The process of claim 4 wherein the unmodified phytase is a phytase from *Aspergillus fumigatus*.

10 6. The process of claim 1 wherein the phytase with the desired property is of eukaryotic origin.

7. The process of claim 6 wherein the phytase with the desired property is of fungal origin.

8. The process of claim 7 wherein the phytase with the desired property is of *Aspergillus* origin.

15 9. The process of claim 8 wherein the phytase with the desired property is a phytase from *Aspergillus terreus*.

10. The process of claim 1 wherein the unmodified phytase is a phytase of *Aspergillus fumigatus* and the phytase with the desired property is the *Aspergillus niger* phytase.

20 11. The process of claim 1 wherein the unmodified phytase is a phytase of *Aspergillus fumigatus* and the phytase with the desired property is the *Aspergillus terreus* phytase.

25 12. A modified phytase with a specific activity improved over the specific activity of the corresponding unmodified phytase wherein the amino acid sequence of the unmodified phytase has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to an amino acid selected from the group consisting of Ala, Val, Leu, Ile, Thr and Asn.

13. The modified phytase of claim 12 wherein the unmodified phytase is the phytase of *Aspergillus fumigatus*.

14. The phytase of claim 13 having additional mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H
5 and N340S.

15. A modified phytase with a specific activity improved over the specific activity of the corresponding unmodified phytase wherein the amino acid sequence of the unmodified phytase has one or more of the following mutations selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L,
10 G277D, G277K, Y282H and N340S.

16. A polynucleotide comprising a DNA sequence coding for a modified phytase of claim 12.

17. A polynucleotide comprising a DNA sequence coding for a modified phytase of claim 13.

15 18. A polynucleotide comprising a DNA sequence coding for a modified phytase of claim 14.

19. A polynucleotide comprising a DNA sequence coding for a modified phytase of claim 15.

20. A vector comprising the polynucleotide of claim 16.

20 21. The vector of claim 20 which is an expression vector.

22 A host cell which has been transformed by a polynucleotide of claim 16.

23. A host cell which has been transformed by a vector of claim 20

24. A food or feed composition comprising a modified phytase of claim 12.
